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NDRI-PR 86-01 JANUARY 1986

SUMMARIES OF RESEARCH FISCAL YEAR 1985



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Naval Medical Research and Development Command Bethesda, Maryland

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SUMMARIES OF RESEARCH Fiscal Year 1985

These summaries cover research carried out from 01 October 1984 through 30 September 1985.

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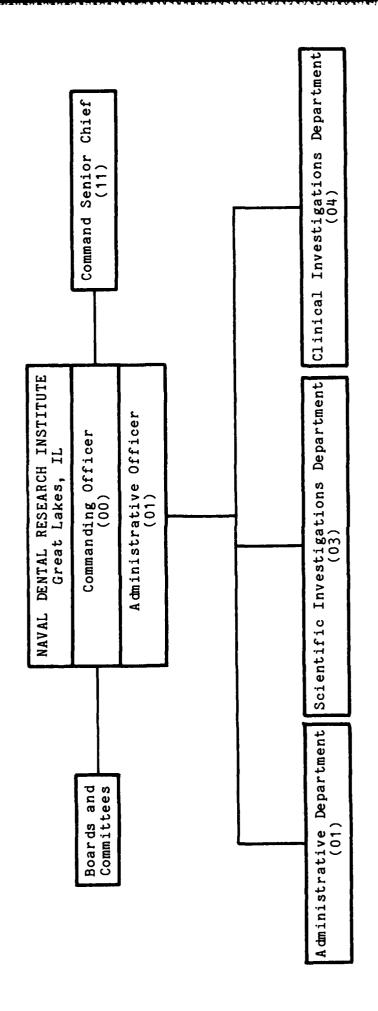
Captain, Dental Corps United States Navy Commanding Officer

TABLE OF CONTENTS

ORGANIZATIONAL CHART	1
COMMAND OVERVIEW	2
STATEMENT OF SIGNIFICANT ACCOMPLISHMENTS	3
ADDENDUM	
WORK UNITS - FISCAL YEAR 1985	A-1
SCIENTIFIC JOURNAL PUBLICATIONS	A-2
RESEARCH PROGRESS REPORTS - Fiscal Year 1985	A-4
FORMAL PRESENTATIONS	A-5
NDRI SEMINAR PRESENTATIONS	A-7
PARTICIPATION IN PROFESSIONAL PROGRAMS AND OTHER MEETINGS	A-8
DISTINGUISHED VISITORS	A-13
HONORS, AWARDS, POSITIONS HELD, CEREMONIES, STAFF ARRIVALS, DEPARTURES AND REENLISTMENTS	A-17



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COMMAND OVERVIEW

COMMAND

The Naval Dental Research Institute was officially established 01 January 1967 with an Officer-in-Charge. The Institute was developed from the Dental Research Facility, which was a Division of the Dental Department of the Naval Administrative Command, Naval Training Center, Great Lakes. The Institute became a fourth echelon command on 17 August 1969. The command is under the direction of the Naval Medical Research and Development Command.

MISSION

The mission of the Institute is to conduct research, development, test and evaluation in dental and allied sciences, with particular emphasis on problems of dental and oral health in Navy and Marine Corps populations and on problems of fleet and field dentistry.

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PERSONNEL

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As of 30 September 1985, there were billets for 12 commissioned officers, 15 civilian employees, and 18 enlisted members, including one U.S. Army Animal Care Technician.

ORGANIZATION

The Institute has undergone reorganization since 1967. current organization of three major Departments is reflected on the preceding page. The Scientific Investigations Department. consists of the Microbiology, Biochemistry/Cell Biology, and Veterinary Sciences/Pathology Divisions. Respectively, they carry out required microbiological, immunological and bacteriological analyses; biochemical studies of etiological agents and of host factors involved in oral disease; assistance, advice and preparation of specimens for histological analysis; and research in the field of laboratory animal medicine and dentistry. Clinical Investigations Department, conducts research related to prevention and treatment of infections, problems of dento-alveolar trauma and injury, and the delivery of optimal dental care for the naval population. The Administrative Department provides the Institute with supply and fiscal services; library, general clerical services and manuscript preparations; photography and graphics; dental equipment repair; and equipment and facility maintenance, as well as special fabrications and instrumentation support.

CLINICAL INVESTIGATIONS DEPARTMENT

Dental emergencies in a military population can have a detrimental influence on military operations and readiness, and can lead to costly medical evacuation procedures. A prototype computer program to assist independent duty corpsmen in the diagnosis and treatment of dental emergencies was completed and field testing begun. This program, a revision of a previous version, is comprehensive in nature and includes five modules: 1) diagnosis of dental emergencies, not trauma-related, 2) diagnosis of dental emergencies, trauma-related, 3) differential diagnosis of soft tissue lesions, 4) definitions of terms, and 5) treatment recommendations. The program can assist in the diagnosis of 35 different dental emergencies and can provide a differential diagnosis on 49 different soft tissue lesions. Preliminary validation was accomplished by a variety of dentists who input over 200 simulated emergencies. The program is undergoing initial testing on actual emergencies at the Naval Dental Clinic, Great Lakes. Concurrent with the development of this program, the contents of a field dental emergency instrument kit continued to be investigated. The instrument kit is being designed for independent duty corpsmen and will be compatible with their training and future resources such as the computer program.

In order to better define and evaluate the magnitude of the dental emergency problem, work on two incidence studies was begun. A "Dental Incident Reporting Log for Independent Duty Corpsmen" was developed to gather information about dental sick call on deployed ships without dental officers. In addition to providing basic epidemiological data, this information will be valuable in evaluating the necessity of providing corpsmen with additional treatment capabilities and in verifying the comprehensiveness of a computerized dental emergency diagnosis system developed at NDRI. A second epidemiological study is being planned to evaluate the cumulative effects of sea duty on oral health. This information would be valuable in directing preventative services. Current efforts are being made to obtain data base support from other naval commands for sample selection and sea duty personnel history information.

Since caries-related dental emergencies constitute an appreciable share of all military dental emergencies, it is essential to have accurate information about trends in treatment delivery. Methods of survival analysis were used to evaluate the time required to treat caries (or defective dental restorations) identified at the initial examination of 506 naval recruits. Nearly 80 percent of carious surfaces were treated during the 18 to 78 month observation period with half the treatment needs being met in the first 6.5 months of service. Deeper lesions were treated earlier and in proportionately greater numbers. Thus, a significant percentage of required treatment is being delivered in

a timely and need appropriate bases. However, almost 80 percent of those surfaces which remained untreated were found in only 15 percent of the total sample. These results suggest that additional efforts to minimize the impact of caries-related dental emergencies should be directed toward treating that small segment of the population who harbor a disproportionately large number of teeth requiring restoration.

A reevaluation of a previously planned study to assess the efficacy of fluoride gels in the arrestment of dental caries was conducted. It was determined that the adverse sequelae of dental caries needed to be better defined. Since the original study proposed that carious lesions be followed over a multi-year period, it was also necessary to determine if carious lesions would remain untreated long enough to be evaluated. The study on caries survival, previously mentioned, determined that a longitudinal approach would not be feasible because most carious lesions were restored within a year-and-a-half. It was also determined that the study as originally planned had a major weakness because it relied heavily on individual patient compliance for application of fluoride gels. The patients to be included in the study would require a high level of cooperation, but unfortunately they would have been selected because they had shown previous dental neglect, a trait that left future compliance requirements in doubt.

In order to reduce emergencies and loss of man hours due to dental problems and to provide an optimum level of dental health, it is essential to have timely, in-depth knowledge of both group and individual dental treatment needs. Epidemiology studies in the past provided some of this information but not efficiently. Data was collected manually, a laborious and error-prone task. process was generally confined to recruit populations, and therefore did not accurately reflect the conditions of deployed or operational personnel. In order to rectify this situation, an automated dental epidemiology system is currently being developed. This system will provide comprehensive and timely dental disease distribution information (automatically) and provide patient dental health status information for both groups and individuals ("flagging" persons with potential dental problems). electronic dental record (including radiographic images) will be a product from which hard-copy dental charts, in standard clinical format, can be regenerated. During the past year, a prototype microcomputer-based dental examination record system was developed and clinically tested on over 200 patients. This system is capable of capturing, storing, and displaying, in standard clinical format, all the information generated during a general The use of this system in forensic dentistry dental examination. was demonstrated following design of a communications software package. Archived electronic dental records were transmitted, via

STATEMENT OF SIGNIFICANT ACCOMPLISHMENTS (Continued)

CLINICAL INVESTIGATIONS DEPARTMENT (Continued)

telephone, within CONUS and overseas following simulated mass casualty disasters. Dental radiographs and other identifying images were digitized into a 512 X 480 X 6 bit array and transmitted across local telephone lines. Finally, a caries-related epidemiology study was conducted using data derived, in part, from the prototype dental examination record system.

Osteocalcin is a non-collagenous bone protein which has been linked to bone metabolism. A study was performed to determine if osteocalcin could be assayed in gingival crevicular fluid to monitor bone loss as a result of periodontal disease. obtaining an antibody for osteocalcin, a non-collagenous bone protein thought to be associated with bone metabolism, numerous immunologic tests were performed. It was determined that this antibody, or the osteocalcin antigen, was unsuitable for testing using an enzyme-linked immunosorbant assay, coagglutination using both cells and latex beads, immunoblotting, immunodiffusion, and avidin-biotin qualitative testing. An <u>in vitro</u> study of gingival crevicular fluid sampling techniques, done in conjunction with this project, determined that the volume and orientation of the pocket to be sampled significantly affects the amount of fluid removed from the pocket. Because the concept of periodontal disease activity is crucial to this and other projects, another closely associated study was performed on the problems involved in determining bursts of periodontal disease activity. It was found that the burst model of periodontal attachment loss is not as strongly founded as originally thought, and that sites having "activity" could be partially, it not totally, due to measurement error.

Because etch-bonded prostheses are, by design, a conservative treatment modality, these devices are delivered with increasing frequency throughout Navy Dental Clinics. Clinical experience with this prosthesis indicates that treatment longevity is a function of etching and bonding quality control. An optical technique for evaluating the extent of etching of the prosthesis retainer was demonstrated. Specifications for a dental laboratory quality control device were prepared and preliminary designs for this device were developed.

Since no definitive statistics relating failures of etch-bonded prostheses exist, it is impossible to accurately assess the cost-effectiveness and develop improvements to the technique. A five year study is underway to evaluate the clinical longevity of 1000 etch-bonded prostheses. This study, to be completed in FY90, was designed to determine the failure incidence, to define the causes of failure, and to recommend refinements for the technique.

Methods to increase the strength of the bond between the tooth and the etched retainer are being analyzed. Ultrasonic energy is being evaluated for its capability to enhance luting resin penetration into etched metal and tooth surfaces. A bond strength test device was designed to overcome the current test drawbacks of tortional and shearing effects distorting the test results.

There is considerable concern among clinicians about transmission of pathogenic organisms via dental instrumentation. Expeditious methods to sterilize dental burs, handpieces, and related instruments in the clinical armamentarium are being investigated. A conventional endodontic glass bead sterilizer was found to inadequately sterilize carbide and steel dental burs. Sterilization capability was found to be improved by using metal beads of a smaller diameter than the conventional glass beads. By layering glass/ceramic beads over the metallic beads, an insulating effect was found to be produced. This effect resulted in an increase in sterilizer temperature and an improvement in the temperature gradient profile, indicating increased sterilization reliability. Microbial sterilization testing is underway.

A survey found that 48 percent of Navy dental officers experienced one or more premature dental bur breakage events. This survey also found that, depending on bur type, premature breakage accounts for 2 to 15 percent of carbide bur usage. The causes of premature bur breakage are being ascertained and will provide recommendations for correction of the problem when completed. The study will determine if the probability of such failures is identifiable by manufacturer lot testing. Scanning electron microscopy indicated that premature bur breakage is due to brittle fracture near the carbide-steel weld. A dynamic test device was designed to test dental burs under simulated clinical use. A static bur test device was developed in collaboration with the American Dental Association. Test device prove-in and test specification development will be completed in FY86.

Similar to the etch-bonded prosthesis, the composite resin bridge offers a conservative interim prosthetic alternative to Navy dental officers who lack convenient access to a prosthetic laboratory. Previous work has shown that failure of this interim prosthesis was via cohesive failure through the body of the composite resin. A mathematical model of prosthesis failure, which was previously developed, was employed to identify the desired mechanical properties and appropriate in vitro tests for composite bridge materials. Experimental resins, compounded using commercial composite resins, were tested and some demonstrated the desired mechanical properties. In addition to use in the composite bridge technique, these materials may find clinical use as temporary restoratives, bonded prosthesis luting agents, and

STATEMENT OF SIGNIFICANT ACCOMPLISHMENTS (Continued)

CLINICAL INVESTIGATIONS DEPARTMENT (Continued)

functional periodontal splint materials. Long-term exposure of commercial composite resins to cultures of normal oral flora showed a negligible decrease in mechanical properties.

The annual clinical evaluation comparing stainless steel crowns with pin-retained amalgam restorations continues to produce longevity data. Preliminary clinical data indicated that when used by a dental officer trained in this technique, the stainless steel crown is an expeditious alternative treatment modality compared to the pin-retained amalgam. Final analysis of the data will be undertaken in FY87.

Maxillofacial wounds constitute at least 15 percent of casualty trauma in combat. The Naval Dental Research Institute has been tasked by Naval Sea Systems Command (NAVSEA) to develop a two-tier ballistic and heat protective maxillofacial shield. This work is proceeding in collaboration with a civilian contractor. The prototype shield is compatible with both the MCU-2/p protective mask with canister hose assembly and the Navy battle helmet. The shield has been designed for incorporation into the NAVSEA Battle Dress Program. Design and initial construction of the shield prototype was completed in FY85. Prototype fabrication and specification testing will be performed in FY86, preparatory to fleet operational testing and evaluation in FY87.

SCIENTIFIC INVESTIGATIONS DEPARTMENT

During FY85, the Microbiology Division has focused on the development of rapid diagnostic methods for detecting microbial indicators of active periodontal disease in military patients. The diagnostic methods are all based on the development of highly specific monoclonal antibodies. Periodontal patients are being used as a model study group for determining patients that could develop dental problems during deployment.

During FY85, monoclonal antibodies to <u>Bacteroides gingivalis</u>, developed during FY84, were fully characterized. The immunoglobulin isotypes for clones III E5/G5, III F8/C2 and VII C2/E2 from fusion BGI were found to all be IgG₁, heavy chains with Kappa light chains. Fusion BG II (whole cell immunogen) hybridomas produced two types of isotype antibodies. Clones V F9/2d, Vf9/4K and Vf9/5a were IgG b heavy chains with Kappa light chains. Clones VI f9/3a, VI f9/3e and VI f9/3i were IgG₁ heavy chains with Kappa light chains. These results were obtained by Enzyme Linked Immunosorbent Assay (ELISA), by Double Immunodiffusion assay and by Radial Immunodiffusion assay against isotype specific antisera. The results also offer a confirmation of the monoclonality of each hybridoma since only one antibody product was produced by each clone.

The specificity of the monoclonal antibodies was studied thoroughly by ELISA using 14 culture isolates of B. gingivalis bacterial whole-cell antigen and 13 non-specific bacterial antigens. The nine clones produced monoclonal antibodies that were found to never cross react with Bacteroides ochraceus, B. maccacae, B. asaccharolyticus, B. melaninogenicus, B. intermedius (6 strains), Fusobacterium sp., and Streptococcus mutans (2 strains). However, six of the nine clones were found to be too specific for certain isolates of \underline{B} . $\underline{gingivalis}$. The \underline{B} . $\underline{gingivalis}$ isolates were obtained from VPI (eight), Forsyth Dental Center (two), U. of Michigan (three) and from ATCC (Type strain 33277). Only the monoclonal antibodies from clones Vf9/2d, 4K and 5a were able to react with the whole cell antigen from all 14 B. gingivalis isolates. The BG-I clones only recognized 7 of 14 isolates and BG-II, VI f9 clones recognized 11 of 14 B. gingivalis antigens. A 1:400 positive polyclonal mouse serum cross reacted with 10 of 13 non B. gingivalis whole cell antigens and reacted with 14 of 14 B. gingivalis isolates. Therefore, the BGII, Vf9 group of clones was completely specific for all B. gingivalis antigens tested and did not cross react with any of the non-B. gingivalis bacterial antiqens.

The sensitivity of the ELISA technique was studied using serial dilutions of whole cell formalin fixed B. gingivalis antigens. The ELISA technique was found to be sensitive enough to detect 4 to 6 g of wet cell antigen.

The three groups of monoclonal antibodies were titered over a dilution range of 1 to 1:160. The cell-free supernatants were found to lose activity rapidly at a 1:10 dilution. Pooled cell-free supernatants were used with 400 μg of B. gingivalis whole cell antigen. Ascites fluids were also titered, and appeared to have greater quantities of antibody present but interference with the ELISA was also noted.

In order to further characterize the monoclonal antibodies, several studies were performed regarding the identity of the B. gingivalis epitopes reacting with each antibody, and the location of the epitopes on the B. gingivalis cell. B. gingivalis MSK extracts were prepared and separated by SDS-polyacrylamide gel electrophoresis (PAGE) and by gradient gel PAGE techniques. Prestained and unstained high molecular weight markers were run with each gel. Gradient gels of 4-30%, 3-27% and 10-20% were used. SDS gels of 8%, 10% and 12% were also used as well as an 8M urea-8% gel protocol. Following separation of the untreated samples (gradient gel PAGE) or SDS treated samples, the separated components were "Western Blotted" to either Nitrocellulose or Zetaprobe paper. The papers were treated with the monoclonal antibodies or control sera after blocking, and developed by an immunoblot peroxidase assay. We found that the epitope which reacts with the monoclonal antibodies appears to be denatured by SDS treatment. When immunoblots of gradient gel separated non-SDS treated cell-free antigens were analyzed, we found that monoclonal antibodies from groups BG II-Vf9 and BG II-VIf9 reacted with epitopes in the range of 65 to 200 KD, whereas monoclonal antibodies from BG-I clones were still unreactive. However, monoclonal antibodies from the BG-I clones may react with antigens too large to enter the gels or may react with uncharged molecules which are not separated and transferred by Western Blot techniques.

Further analysis of the monoclonal antibody reactions involved the interaction of mixtures of the antibodies and whole cell B. gingivalis antigens. When BGI III E5 was mixed with BG II Vf9, an enhanced ELISA O.D. resulted. However, when BGII, Vf9 and BGII VIf9 were mixed, some interference was observed since a lesser ELISA O.D. value resulted, when compared to either of the antibodies alone. A mixture of BGI III E5 and BGII VIf9 also resulted in an enhanced ELISA O.D. A mixture of all 3 groups of antibodies resulted in a somewhat enhanced reaction relative to the BGII antibody groups, but was not as strong as some of the BGI with BGII mixtures. These results indicate that the antigenic epitope target of BGI clone antibodies is distinct from that of the BGII clones. However, the two groups of BGII appear to produce monoclonal antibodies to similar or overlapping, although not identical, epitopes since the interference is incomplete.

We performed several experiments using the electron microscope in an attempt to visualize the antiquenic targets of the monoclonal antibodies on the B. gingivalis cell. In one study, a goat anti-mouse peroxidase label was employed to locate the reaction site of the monoclonal antibodies. Polyclonal mouse serum was used as a positive control and either negative serum or a buffer-negative control. This stain was performed after embedding and sectioning B. gingivalis cells. The results were inconclusive due to non-specific staining of the epon. A ferritin-labelled rabbit anti-mouse reagent was used with similar results. ferriti -anti-mouse IgG complex was prepared using an FNPS cross-linking agent, and stained both pre- and post-embedding. Again non-specific staining resulted. A hemocyanin-labelled anti-mouse reagent was also prepared. The label appeared quite distinct when viewed by transmission electron microscopy. However, again it was found to be nonspecific since negative control cells also had the label.

Various reagents such as peroxidase and ferritin-labelled specific anti-mouse IgG preparations were used prior to embedding and sectioning, followed by uranyl acetate and lead citrate A new ferritin-anti ferritin technique was also used. Unbound ferritin was difficult to remove from the cells, and non-specific staining was still observed. Protein A-gold and qoat-anti mouse-qold labels were also tested. They could not be used in pre-embedding techniques since they could not be separated from the heavy cells. Both gold labels were also found to be non-specific when used in post-sectioning procedures. Epon-sectioned cells were treated with peroxide and an alkaline etching agent to improve immunoreactivity. The results were not suitable since non-specific staining was still observed and overetching resulted in a loss of physical support. The results were not sufficient to visualize the site of antigen-antibody reaction, although there was some indication that the BG-II monoclonal antibodies were all directed at cell surface antigens (or capsule antigen).

Fluorescent antibody studies were also used in defining the specificity of the monoclonal antibodies. The results exactly mirrored the ELISA results, although the indirect fluorescence procedure is a qualitative method.

During FY85 it was found that cryopreserved cells need to be thawed rapidly and cultured for growth on a feeder layer of rat spleenocytes since less than 20% of the cells remain viable. We have also started examining clinical subgingival plaque samples stored in formalinized saline solution. A new microfiltration assay as well as ELISA to evaluate the quantity of B. gingivalis antigen present in these samples will be used. Early results using samples from young naval personnel indicated low levels of

B. <u>qingivalis</u> antigen. Samples will be assayed of subgingival plaque obtained from an older population (aged 25-50 years) being treated at Northwestern University and an attempt to correlate B. <u>qingivalis</u> content with pocket depth will be made.

The first monoclonal antibody produced was against the periodontopathic organism, B. gingivalis. Once the organisms that are also related or associated with specific periodontal diseases are determined, we will concentrate on producing monoclonal antibodies against these organisms. The monoclonal antibodies will then be used for the rapid identification of these organisms and should aid in the diagnosis and in monitoring treatment of periodontal diseases found in naval personnel. By facilitating diagnosis and definitive treatment during non-critical periods, such as port-calls and overhaul periods, these diagnostic aids could lessen the risk of acute episodes of disease which threaten operational effectiveness and mission readiness.

After a monoclonal antibody to <u>B. gingivalis</u> was devloped and characterized in our laboratory, it was used in a coagglutination (CoA) procedure for the rapid identification of <u>B. gingivalis</u>. If the procedure used, Protein A, a cell-wall fraction of <u>Staphylococcus aureus</u>, was bound to the monoclonal antibody. When this reagent is mixed with an antigen, if specific for the antigen, an agglutination reaction is visible (on a glass slide) within 1-2 minutes. The reagent was tested against a variety of Bacteroides and other periodontopathic organisms; only <u>B. gingivalis</u> strains gave a positive response or an agglutination reaction.

In testing for the sensitivity of the CoA test, 10 known B. gingivalis strains were grown and 50 mg/ml of formalinized wet weight of cells for each strain was prepared. These cells were serially diluted and tested against 50 ul of the CoA reagent. The greatest dilution of the antigen that still gave a positive response with the CoA reagent ranged from 78-625 ug/ml. Seven of the ten strains agglutinated in the 78-312 ug/ml range of antigen. These results indicate the excellent sensitivity of this test.

The CoA test was then used in a clinical study to evaluate the test by comparing it with the physiological and biochemical procedures normally used to identify B. gingivalis. Gingival pocket samples were collected with a curette, from 217 male naval recruits aged 17-28 years of age and diagnosed as having periodontitis with gingival pocket depths ranging from 3-9 mm. The samples were grown on Wilkins-Chalgren agar and representative brown to black colonies were picked, purified, and characterized. From the 217 clinical samples, only 11 (5.0%) organisms were biochemically positive as B. gingivalis. These 11 samples were the only ones that also gave a positive agglutination reaction with the CoA test.

The use of the <u>B. gingivalis</u> monoclonal antibody in the CoA test proved to be very specific, rapid, and an easy-to-use method for distinguishing <u>B. gingivalis</u> from other Bacteroides strains. The use of the CoA procedure could be adapted from the rapid identification of other microorganisms in diseased tissue of naval personnel.

During FY85, the Microbiology Division has also studied the virulence factors of Streptococcus mutans. S. mutans is the major etiologic agent of dental caries. Its cariogenicity has been associated with its ability to produce lactic acid and water-insoluble glucans. The water-insoluble glucans may be important in the initiation of caries due to their ability to aggregate S. mutans on tooth surfaces and to act as a diffusion barrier to the lactic acid produced in dental plaque. This study related both of these virulence factors from the same organism to caries activity in laboratory animals. The results obtained could help determine the role and importance of each factor in the caries process.

Sixteen S. mutans strains, that synthesized various levels of lactic acid and water-insoluble glucans, were sent to Ohio State University for testing in germfree rats. Each strain was implanted into 10 gnotobiotic animals, and the relationship of caries development to the synthesized amounts of lactic acid and water-insoluble glucans was determined. The organisms were reisolated at the termination of the animal phase of the study; the lactic acid and water-insoluble glucans levels were again determined to see if the virulence factors changed while the organism was in the test animals.

When the rats were sacrificed they were evaluated for caries by the method of Keyes. In the third and fourth experiments, the levels of S. mutans were determined by removing the molars of one quadrant, macerating in a tissue homogenizer, and plating. were analyzed by ANOVA, Newman-Keuls, and correlation analysis. There was no significant correlation between buccal-lingual and total severity caries scores and levels of glucan, lactate, or levels of S. mutans. A significant positive correlation was found between lactate production and proximal caries (p < 0.001). reinforces the importance of acidogenicity of microorganisms as a virulence factor for dental caries. This does not negate the importance of insoluble glucans in the caries process; instead, the lack of correlation between glucan and buccal-lingual caries suggests that the hamster model, rather than the rat model, should also have been considered. In the hamster caries-model, the level of insoluble glucan present appears to be related to caries activity.

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The test data on the rat experiments can be seen in Table 1.

INSOLUBLE GLUCAN, LACTIC ACID AND CARIES ACTIVITY OF SELECTED STRAINS OF STREPTOCOCCUS MUTANS TABLE 1

1 1 1 1	Gluce	5	Glucan (mg/mg DNA)		Lactic Acid	ic Acid	Me.	Mean Caries Scores in Gnotobiotic Rats*	Scores Rats*		S. mitans
strain	Soluble Pre Post		Insoluble Pre Post	~ ∤	µ moles/ Pre	hg DNA Post	Proximal	Buccal- Lingual	Total Severity	- Serotype	No. x 10 / quadrant
Dowd Stacey Ward Gunn	1 89 1 98 1	13 138 107 113	70 12 19	160 8 34 19	2.0	3.0 2.2 1.8 2.2	1.9	8.1 7.6 1.2 2.6	104 96 104	0 0 6/p	
107B OMZ 175 Thresher HS-6	143 25 39 49	36 11 26 51	5 15 25	26 3 26	1.7	2.3	0.3** 2.6 2.3 1.8	8.8 7.0 12.3 11.4	94 103	മാധര	
Parker Silver Von Wick Clark	91 85 122 1 63	96 108 59	18 12 26 13	7 46 12	3.1 3.8 2.8 8.2	3.5 3.5 3.7		7.88.2 5.8.6 5.8.6	36 34 34	0000	110 130 130 198
130P (light) 130P (dark) Tea Sink Ford	87 71 4	256 12 6 6 5	1 78 30 51	2 44 43 43	2.7 3.1 3.4	4.8 5.3 7.4 7.7	6.9 12.8 13.0 13.5	2.1 4.1 3.9 0.9	54 50 50 50 50	9/p 9/p 9/p	221 124 239 167

*Values within lines are not significantly different. **107B significantly different from OMZ 175 and Thresher. Each block of four lines represents a separate experiment. In the latter 2 blocks, one upper and one lower quadrant were scored for caries. In the first 2 blocks, all 4 quadrants were scored.

There were 10 rats/organism in the first 3 blocks and 8 rats/organism in the fourth block.

STATEMENT OF SIGNIFICANT ACCOMPLISHMENTS (Continued)

SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

It can be concluded that, with additional work, appropriate preventive and treatment procedures could be developed to control the virulence factors of <u>S. mutans</u>, with the subsequent reduction of new carious lesions in naval personnel. However, at this time, due to changes in research priorities, and funding and personnel constraints, this project is now terminated.

The Biochemistry/Cell Biology Division has conducted several studies this past year on developing an in vitro alveolar bone model metabolic system, as well as studying fibronectin in the oral cavity. Fibronectin (FN) is a glycoprotein that functions as a non-specific opsonin, that promotes adhesion of fibroblasts to collagen, and influences the attachment of bacteria to soft tissues.

As a first step, the FN assay method, an ELISA procedure, was modified to improve its accuracy and reproducibility. Following review of assay protocols, the primary change introduced was to coat the wells of the assay plates with 1% gelatin, then to bind the gelatin to the plates with 0.5% glutaraldehyde. This modification yielded much more reproducible data with unstimulated saliva samples than several other procedural variations because FN binds strongly to gelatin. Assays were then conducted on moderately stimulated and unstimulated saliva samples that had been collected as part of an earlier study and maintained at -80 C prior to the FN assays. Data, expressed as Mean + S.D. for each measurement, are shown in the Table.

COMPARISON OF SALIVARY FIBRONECTIN LEVELS FOR CARIES-FREE AND CARIES-ACTIVE RECRUITS

Subjects	N	Unstimulate	ed Saliva	Moderately Sti	m. Saliva
Caries-Free Caries-Active		Flow Rate* 0.38+0.31 0.36+0.18	FN** 192+252 207+197	Flow Rate 0.67+0.50 0.69+0.32	FN 140+127 368 <u>+</u> 335

The intergroup difference in FN levels for the stimulated samples suggested that stimulation might result in contamination of samples by minute amounts of blood (plasma FN level = 300 ug/ml), particularly for subjects with poor oral health. Unstimulated samples would then appear to be preferable in further studies involving the potential role of fibronectin as a diagnostic indicator of periodontal disease.

Salivary FN levels were then investigated in subjects with and without periodontal disease, after 10-minute unstimulated saliva samples had been collected from approximately 75 periodontaldiseased (PD) and about 55 PD-free recruits. This study assumed that unstimulated saliva would contain some gingival fluid, following passage of the saliva across the gingivae during collection. Since gingival fluid may be considered an exudate of blood plasma, the FN levels could be 1000x or greater than those commonly found for saliva. The salivary FN levels of PD subjects might then be expected to be much higher than those for PD-free Analyses were conducted on samples from 20 PD-free subjects (mean age, 20.5) and from 20 PD subjects (mean age, Ten of the PD subjects had localized juvenile periodontitis, while the disease status of the remaining subjects ranged from moderate to severe periodontitis. Calculations of salivary flow rates (mean \pm s.d.) showed 0.31 \pm 0.21 ml/min for the PD group and $0.37 + 0.\overline{2}1$ ml/min for the PD-free group; these values were not significantly different. FN results, using the ELISA method, showed 376 + 838 ng/ml for the PD group compared to 107 + 123 ng/ml for the PD-free group. However, the PD group mean was strongly skewed by two subjects, whose FN levels were each over 6x higher than the group mean. The salivary FN for the remaining 18 subjects was 108 + 107 ng/ml, essentially the same as for the PD-free group. It was concluded that, under conditions of the study, no correlation of PD status and salivary FN content could be established, and the potential role of FN as a diagnostic indicator of periodontal disease could not be demonstrated.

Another study explored the effects of various tooth-root treatments on enhancing attachment of FN to tooth-root surfaces. FN is probably the most important component of connective tissues, after collagen and elastic fibers. With its numerous binding sites, FN is an ideal substance for linking a variety of extracellular components with cells, forming a harmonious whole. Along with attaching cells to substrates such as collagen, FN forms a scaffold with fibrin, providing a lattice work for fibroblast and endothelial cell migration, orientation, and growth. These interactions may be particularly important in the initial fibroblast reattachment to tooth-roots that is needed, following periodontal treatment, for the formation of new connective tissues.

Groups of planed and unplaned root sections were aciddemineralized in order to expose as much collagen, which is part of the root structure, as possible. These sections were then treated with tritiated FN and the amount of label per square millimeter of root surface was measured. To determine the importance of the structural collagen, planed and unplaned surfaces, some of which were acid-demineralized, were exposed to collagenase before and after FN treatment.

STATEMENT OF SIGNIFICANT ACCOMPLISHMENTS (Continued)

SCIENTIFIC INVESTIGATIONS DEPARTMENT (Continued)

The unplaned acid demineralized root surfaces were capable of consistently binding the highest levels of FN. Treating any of the prepared surfaces with collagenase significantly reduced the adherence of FN; this strongly supported recent reports that have found collagen to be an important factor in the attachment of FN to tooth-root surfaces.

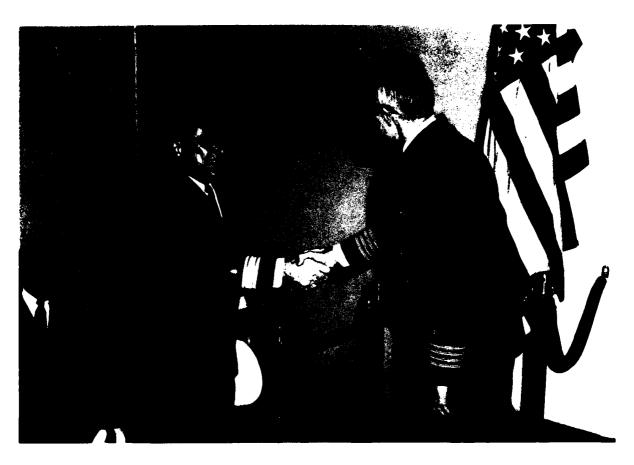
The single most important characteristic of periodontal disease, when tooth loss is considered, is the breakdown of the periodontal attachment apparatus of which alveolar bone is an integral component. The mechanism of the loss of alveolar bone, which occurs following the inflammation of the surrounding periodontal tissue, is not well understood.

An in vitro model system based on cultured cells provides the investigator with route for the study of alterations in bone cell metabolism. In vivo systems, while not without their advantages, offer some disadvantages not seen with cultured cells. In vivo models contain a variety of cell-types which interact thereby producing a potentially realistic but exceedingly complicated and poorly controlled environment for experimentation. Dissociated cell culture, on the other hand, permits the development of a simpler experimental system with a substantial reduction in the number of uncontrolled factors. The simplification provides the investigator with greater opportunities to study the metabolism of osteoblasts and the biological factors that modulate their behavior.

Human alveolar bone specimens were obtained from the Naval Dental Clinic and were used to develop a method for explanting alveolar bone-derived cells to cell culture. Initial attempts using sequential collagenase digestions proved unsuccessful so the bone was explanted directly to culture after mincing into fragments less than 1 mm³. Within one week of explantation, cells were seen emerging from the bony matrix and attaching to the plastic substrate. These cells proved amenable to culture in conventional serum-supplemented medium. Once a sufficient number of cells emerged, the cells were passed and the bone chips removed from the system. The cell strains derived from several explants have been successfully passed in serial culture.

Once stable cell lines were established, a method for adapting the cells to a test system allowing DNA synthetic and mitotic rates was developed. By subjecting the cells to serum-starvation, the cells are brought to reproductive quiescence then stimulated with the particular bioactive agent of interest. If the agent is capable of inducing DNA synthetic or mitotic rate changes, alterations in H-thymidine uptake and cell number are noted. Before such a system proved workable, parameters involving initial cell seeding numbers and length and intensity of serum-starvation were established.

Once an in vitro assay system was perfected, the effects of some bioactive agents were analyzed. These initial studies indicated that epidermal and fibroblast growth factors (EGF, FGF) both induced increases in the DNA synthetic rate. Longer term growth studies, on the other hand, indicated that beta-glycerophosphate and ascorbic acid induce no visible microscopic morphologic changes, osteoid production or mineralization. Having established a workable in vitro system, studies involving the electrophysiology, ion exchange characteristics and hormone sensitivities of the cells are planned.



Captain G. E. Clark greeting COMO R. G. Shaffer, DC, USN, Chief Navy Dental Corps.

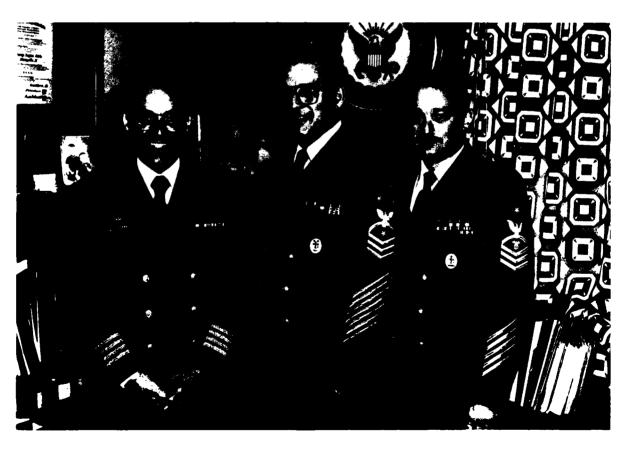
ADDENDUM TO

SUMMARIES OF RESEARCH - Fiscal Year 1985

- 61153N MR0412002-0445 The Pathogenesis of Oral and Dental Diseases
- 63706N M0095003-3028 Evaluation of New Methods and Materials to Arrest Oral Disease, and to Prevent and Treat Dental Emergencies in Naval Personnel

INDEPENDENT RESEARCH WORK UNITS

- 61152N MR000.01.01-0042 Evaluation of Dacron Reinforced Silastic as a Replacement for the Temporomandibular Joint Meniscus
- 61152N MR000.01.01-0050 Role of Oral Microflora in the Chemical Degradation of Dental Luting Resins
- 61152N MR000.01.01-0051 Cellular Interactions and Stability of Fibronectin in the Oral Cavity
- 61152N MR000.01.01.0053 Osteocalcin and Osteonectin as Markers of Bone Metabolism in Periodontal Disease
- 61152N MR000.01.01-0054 <u>In vitro Model for the Study of Human Alveolar Bone</u>



Captain G. E. Clark, HMCM L. V. Green (Force Master Chief, Naval Medical Command) and DTCS M. G. Hastings.

SCIENTIFIC JOURNAL PUBLICATIONS

- Cohen, M. E. Alpha Level Distortions with Repeated Testing of Follow-up Dental Data. Journal of Dental Research 63:1214-1216, 1984.
- Cohen, M. E. Dental Anxiety and DMFS Status: Association within a US Naval Population versus Differences Between Groups. Community Dentistry and Oral Epidemiology 13:75-78, 1985.
- Cohen, M. E., Walter, R. G., Hyman, J. J., and Tombasco, P. K. Age-Specific Angulation of Unerupted Human Third Molar Teeth in a Cross-sectional Sample. Archives of Oral Biology 30:441-444, 1985.
- Diehl, M. C. Open Wide and Say Computerize Touch Screen Automated Dental Examination. Medicine and Computer, January 1985.
- Hyman, J. J. and Cohen, M. E. The Predictive Value of Endodontic Diagnostic Tests. Oral Surgery, Oral Medicine, Oral Pathology 58:343-346, 1984.
- Kelly, J. R. Surface Structures of Etched Nickel-Based Dental Casting Alloys Characterized by Reflection Photometry. A thesis submitted to the Faculty of the Graduate School, Marquette University in partial fulfillment of the requirements for the Degree of Master of Science, Milwaukee, Wisconsin, May 1985.
- Lamberts, B. L., Pruitt, K. M., Pederson, E. P., and Golding, M. P. Comparison of Salivary Peroxidase System Components in Caries-Free and Caries-Active Naval Recruits. Caries Research 18:488-494, 1984.
- Lamberts, B. L., Pederson, E. P., and Simonson, L. G. The Effects of Basic and Acidic Synthetic Polypeptides on the Adherence of the Oral Bacteria, Streptococcus mutans and Streptococcus sanguis, to Hydroxyapatite. Archives of Oral Biology 30:295-298, 1985.
- Ralls, S. A. and Marshall, E. Dental Management of a Patient with Glycogen Storage Disease Type I. Journal of the American Dental Association 110:723-726, 1985.
- Ralls, S. A. and Warnock, G. R. Stomatitis Areata Migrans Affecting the Gingiva. Oral Surgery 60:197-201, 1985.
- Rosen, S., Shklair, I. L., Beck, E. X., and Beck, F. M. Virulence Factors of Streptococcus mutans. In: Germfree Research: Microflora Control and Its Application to Biomedical Science, pp. 211-215, Alan R. Liss Inc. Publishers, 1985.

Southard, T. E., Baycar, R. S., and Walter, R. G. Forensic Dentistry: Electronic Transmission of Computerized Records. Military Medicine 150:492-495, 1985.

Southard, T. E. Storage of Radiographic Images via Laser Optical Disk: A Preliminary Study. Oral Surgery, Oral Medicine and Oral Pathology 60:436-439, 1985.

Southard, T. E. A Microcomputer-Based Dental Epidemiology System. Proceedings of the Seventh Annual Conference of the IEEE Engineering in Medicine and Biology Society, pp. 874-876, 1985.



Captain D. E. Furry presenting DT1 W. F. Bruton with a plaque and Letter of Commendation for selection as the Naval Medical Research and Development Command Sailor of the Year nomination.

RESEARCH PROGRESS REPORTS - FY 1985

NDRI-PR 84-08	Alpha Level Distortions with Repeated Testing of Follow-up Dental Data
NDRI-PR 84-09	Comparison of Salivary Peroxidase System Components in Caries-Free and Caries-Active Recruits
NDRI-PR 85-01	Summaries of Research - Fiscal Year 1984
NDRI-PR 85-02	An Automated Information System: Results After Four Years
NDRI-PR 85-03	The Predictive Value of Endodontic Diagnostic Tests
NDRI-PR 85-04	Stability of Epinephrine in Dental Anesthetic Solutions: Implications for Autoclave Sterilization and Elevated Temperature Storage
NDRI-PR 85-05	The Effects of Basic and Acidic Synthetic Polypeptides on the Adherence of the Oral Bacteria, Streptococcus mutans and Streptococcus sanguis, to Hydroxyapatite
NDRI-PR 85-06	Dental Anxiety and DMFS Status: Association within a US Naval Population Versus Differences Between Groups
NDRI-PR 85-07	Age-Specific Angulation of Unerupted Human Third Molar Teeth in a Cross-Sectional Sample
NDRI-PR 85-08	Optical Surface Roughness and Selective Etching of Interdendritic Rexillium III Phase(s)

FORMAL PRESENTATIONS MADE AT MEETINGS OF SCIENTIFIC SOCIETIES/GROUPS

NOVEMBER

CLARK, G. E., presented "Treatment of Deep Carious Lesions", at G. V. Block Lecture Series, Ohio State University Dental School, Columbus, Ohio.

SIMONSON, L. G., presented "Monoclonal Antibodies as Oral Disease Diagnostic Agents" at Illinois State University, Normal, Illinois.

FEBRUARY

BAYCAR, R. S., presented a lecture on forensic dentistry to the medical personnel of the Great Lakes Reserve Unit, Great Lakes, Illinois.

SOUTHARD, T. E., presented "Computerized Dental Examination Record System" at the American Dental Association Midwinter Meeting, Chicago, Illinois.

MARCH

The following presentations were given at the 63rd General Session of the International Association for Dental Research in Las Vegas, Nevada:

- COHEN, M. E., "Dental Treatment Anxiety as a Predictor of Treatment Demand"
- ESQUIRE, R. G., "Doubly Multivariate Analysis of Oral Microbial Changes in Navy Personnel"
- KELLY, J. R., "Optical Surface Roughness and Selective Etching of Interdendritic Rexillium III Phase(s)"
- LAMBERTS, B. L., "Trypsin-Like Activities of Bacteroides gingivalis During 7-Day Culture Periods"
- PEDERSON, E. D., "Susceptibility of Fibronectin to Degradation by Various Oral Microorganisms"
- SHKLAIR, I. L., "Diagnostic Value of a Coagglutination Procedure Using Monoclonal Antibodies"
- SIMONSON, L. G., "Production and Characterization of Monoclonal Antibodies to Bacteroides gingivalis"

ESQUIRE, R. G., presented "Doubly Multivariate Analysis of Oral Microbial Changes in Naval Personnel" at the American Association for Dental Research Chicago Section, Loyola University, Chicago, Illinois.

FORMAL PRESENTATIONS MADE AT MEETINGS OF SCIENTIFIC SOCIETIES/GROUPS (Continued)

MARCH (Continued)

SIMONSON, L. G., presented "Production and Characterization of Monoclonal Antibodies to <u>Bacteroides gingivalis</u>" at Loyola University Dental School, Chicago, Illinois.

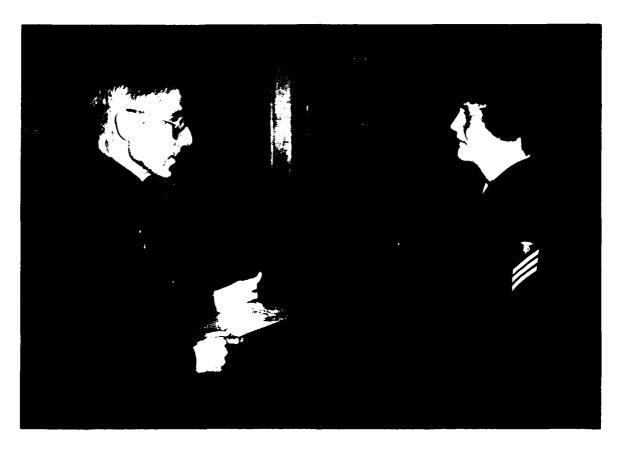
MAY

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HETZER, M. T., presented a table clinic "AIDS: Identification and Prevention of Risk" at the Great Lakes Dental Society Meeting, Great Lakes, Illinois.

SEPTEMBER

SOUTHARD, T. E., presented "Microcomputer-Based Dental Epidemiology System" at the 7th Annual IEEE/EMBS Frontiers of Engineering and Computing in Health Care, Chicago, Illinois.



Captain G. E. Clark presenting DN L. A. Rouse with a Navy Achievement Medal.

NDRI SEMINAR PRESENTATIONS FOR GREAT LAKES AREA NAVAL DENTAL OFFICERS

NOVEMBER

CLARK, G. E., presented "Treatment of Deep Carious Lesions".

SIMONSON, L. G., presented "Use of Monoclonal Antibodies and Diagnosis of Periodontal Disease".



Captain R. G. Esquire presenting Mr. John H. Ringgold with a certificate and pin for 25 and 30 years of Federal Service.

OCTOBER

CLARK, G. E., attended the American Dental Association Annual Meeting in Atlanta, Georgia.

The Chicago Section of the American Association for Dental Research meeting was attended by the following personnel:

BAYCAR, R. S. ESQUIRE, R. G. LAMBERTS, B. L.

BAYCAR, R. S., attended a working conference at Scott Aviation, Monrovia, California.

NOVEMBER

The Great Lakes Dental Society meeting was attended by the following personnel:

BAYCAR, R. S. ESQUIRE, R. G. HETZER, M. T.

DECEMBER

ESQUIRE, R. G., attended the Combined Federal Campaign Awards Ceremony as the Naval Dental Research Institute representative.

JANUARY

The Chicago Section of the American Association for Dental Research meeting was attended by the following personnel:

BAYCAR, R. S. ESQUIRE, R. G. HETZER, M. T. SIMONSON, L. G.

The Great Lakes Dental Society meeting was attended by the following personnel:

BAYCAR, R. S. CLARK, G. E. ESQUIRE, R. G. HETZER, M. T.

HASTINGS, M. G., attended a basic procurement course conducted by the General Services Administration, Chicago, Illinois.

JANUARY (Continued)

HETZER, M. T., attended a lecture on resin bonded bridges at the Naval Dental Clinic, Great Lakes, Illinois.

SEROWSKI, A., attended a facial shield review at Scott Aviation, Monrovia, California.

FEBRUARY

The Ballistic Face Shield Design Conference at Naval Sea Systems Command, Alexandria, Virginia, was attended by the following personnel:

BAYCAR, R. S. SEROWSKI, A.

The Chicago Dental Society Midwinter meeting was attended by the following personnel:

BAYCAR, R. S. KELLY, J. R. CLARK, G. E. RALLS, S. A. ESQUIRE, R. G. SOUTHARD, T. E. HETZER, M. T.

CLARK, G. E., attended a meeting of the American College of Dentists, Chicago, Illinois.

CLARK, G. E., attended a meeting of the International College of Dentists, Chicago, Illinois.

REESE, W. V., attended the Congress for Health Care Administrators, Chicago, Illinois.

MARCH

BAYCAR, R. S., attended a Design Conference for Ballistic Face Shield, Scott Aviation, Monorovia, California.

CLARK, G. E., assisted in presentation of NDRI Dental Emergency Diagnosis Program to Chief of Army Dental Corps, the Pentagon, Washington, D. C.

The 63rd General Session of the International Association for Dental Research, Las Vegas, Nevada, was attended by:

CLARK, G. E. JONES, T. R.
COHEN, M. E. LAMBERTS, B. L.
ESQUIRE, R. G. PEDERSON, E. D.
HETZER, M. T. RALLS, S. A.
KELLY, J. R. SHKLAIR, I. L.
SIMONSON, L. G.

MARCH (Continued)

CLARK, G. E., attended the American Association for Dental Research Chicago Section meeting, Chicago, Illinois.

REESE, W. V., attended the Practical Comptrollership Course, Naval Postgraduate School, Monterey, California.

SEROWSKI, A., attended facial shield review, Monrovia, California.

APRIL

The Chicago Section of the American Association for Dental Research meeting was attended by the following personnel:

BAYCAR, R. S. CLARK, G. E. SIMONSON, L. G.

ESQUIRE, R. G., attended Endurance Physiology Symposium, American Medical Joggers Association, Boston, Massachusetts.

HASTINGS, M. G., attended the retail inventory and financial management course sponsored by the General Services Administration, Chicago, Illinois.

JONES, T. R., attended the MEDLARS User Instruction Course for Health Professionals, University of Illinois, Library of the Health Sciences, Chicago, Illinois.

SEROWSKI, A., attended the Society of the American Military Engineers, Great Lakes, Illinois.

MAY

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BAYCAR, R. S., attended the Operating Forces Management Seminar, Naval Dental Clinic, Bethesda, Maryland.

The Chicago Section of the American Association for Dental Research meeting was attended by the following personnel:

CLARK, G. E. ESQUIRE, R. G. HETZER, M. T. LAMBERTS, B. L.

The Great Lakes Dental Society meeting was attended by the following personnel:

CLARK, G. E. ESQUIRE, R. G. HETZER, M. T.

MAY (Continued)

COHEN, M. E., attended a conference on Clinical Trials in Periodontal Diseases, Arlington Park, Illinois.

JUNE

The following personnel attended training on computer software for the Zenith 120:

BAYCAR, R. S.
CLARK, G. E.
COHEN, M. E.
ESQUIRE, R. G.
GAMBLE, R. W.
HETZER, M. T.
HARMON, S. R.
JONES, T. R.
JOHNSON, M. J.
KLINE, S. J.
MCKENDALL, G. M.
PORTIS, M. J.
QUIRING, R. C.

RALLS, S. A.
REESE, W. V.
ROBERSON, R. R.
ROUSE, R. F.
TUTEN, E. L.
SEROWSKI, A.
SHKLAIR, I. L.
SIMONSON, L. G.
SMITH, H. E.
STEWART, S. P.
TOMBASCO, P. K.
TUCKER, D. A.
TUTEN, E. L.

SIMONSON, L. G., attended the Illinois Society for Microbiology spring meeting, Chicago, Illinois.

JULY

A Design Presentation Conference on Ballistic Face Shield, Naval Sea Systems Command, Crystal City, Virginia was attended by the following:

BAYCAR, R. S. SEROWSKI, A.

A Naval Dental Research Institute Epidemiology/ADP Program Review/Consultation with the staffs of the Naval Health Research Center and Naval Hospital, San Diego, California, was attended by the following personnel:

CLARK, G. E. RALLS, S. A.

HASTINGS, M. G., attended a budget execution class sponsored by the General Services Administration, Chicago, Illinois.

JONES, T. R., attended the Gordon Conference on Bones and Teeth in Meriden, New Hampshire.

REESE, W. V., attended the medical orientation class on substance abuse at the Naval Hospital, Great Lakes, Illinois.

JULY (Continued)

The following personnel attended training on the Lotus 1, 2, 3 software:

CLARK, G. E.
GAMBLE, R. W.
HASTINGS, M. G.
KLINE, S. J.
ROUSE, R. F.
SIMONSON, L. G.
STEWART, S. P.

SEPTEMBER

CONTRACTOR SECRETARIES OF SECRETARIE

The Great Lakes Dental Society meeting was attended by the following personnel:

ESQUIRE, R. G. HETZER, M. T.

HETZER, M. T., attended the Gorham Conference on The Use of Coupling Agents in Polymeric Composites in Monterey, California.

DISTINGUISHED VISITORS

OCTOBER

Captain E. B. Hancock, DC, USN, Oral and Dental Health Program Manager, Naval Medical Research and Development Command, Bethesda, Maryland.

Colonel C. W. DeLannoy, USAR, VC, Department of Animal Medicine, Michael Reese Hospital, Chicago, Illinois.

Captain I. L. Rubin, USA, MEDDAC, Fort Sheridan, Illinois.

SP5 H. W. Malcolm, USA, MEDDAC, Fort Sheridan, Illinois.

Commander J. C. Cecil III, DC, USN, Naval Dental Clinic, Norfolk, Virginia.

NOVEMBER

Colonel T. P. Sweeney, DC, USA, Commanding Officer, U.S. Army Institute of Dental Research, Walter Reed Army Medical Center, Washington, D. C.

Lieutenant Colonel L. S. Kopelman, U.S. Army Institute of Dental Research, Walter Reed Army Medical Center, Washington, D. C.

Captain R. P. Whitlock, DC, USN, Naval Medical Command, Washington, D. C.

Captain R. G. Ireland, MC, USN, Naval Medical Research and Development Command, Bethesda, Maryland.

Dr. H. R. Lucien, National Research Council, Washington, D. C.

Mr. C. E. Williams, National Research Council, Washington, D. C.

Dr. J. F. Volker, National Research Council, Birmingham, Alabama.

Mr. D. C. Hindo, Veterans Administration Medical Center, North Chicago, Illinois.

Mr. B. H. Youn, Veterans Administration Medical Center, North Chicago, Illinois.

Captain I. L. Rubin, USA, MEDDAC, Fort Sheridan, Illinois.

Colonel C. W. DeLannoy, USAR, VC, Department of Animal Medicine, Michael Reese Hospital, Chicago, Illinois.

DECEMBER

Captain I. L. Rubin, USA, MEDDAC, Fort Sheridan, Illinois.

SP5 H. W. Malcolm, USA, MEDDAC, Fort Sheridan, Illinois.

Comodore W. J. Sears, Chief of Naval Operations Officer, OPNAV093, Washington, D. C.

Captain H. H. Sowers, Commanding Officer, Naval Hospital, Great Lakes, Illinois.

JANUARY

Captain I. L. Rubin, USA, MEDDAC, Fort Sheridan, Illinois.

Captain S. A. Muller, MC, USN, Executive Officer, Naval Hospital, Great Lakes, Illinois.

FEBRUARY

Dr. S. Hoff, Chief, Pharmacology Department, Chicago Medical School, North Chicago, Illinois.

Captain R. B. Doremus, Commanding Officer, Personnel Support Activity, Great Lakes, Illinois.

Commander R. L. Finke, USN, Commanding Officer, Naval Drug Screening Lab, Great Lakes, Illinois.

Major Debok, USA, Fort Leonard Wood, Missouri.

Captain R. H. Wyttenbach, USN, Commanding Officer, Service School Command, Great Lakes, Illinois.

Captain B. J. Suse, USN, Commanding Officer, Administrative Command, Great Lakes, Illinois.

Captain N. C. Lord, USN, Commanding Officer, Recruit Training Command, Great Lakes, Illinois.

Mr. M. Duoma, Massachusettes Manufacturing Company, Cambridge, Massachusetts.

Commodore R. M. Shaffer, Naval Medical Command, Washington, D. C.

Captain G. M. McWalter, DC, USN (Ret.), University of Texas, San Antonio, Texas.

Captain E. H. Plump, DC, USN, Commanding Officer, Naval Dental Clinic, Great Lakes, Illinois.

MARCH

- Colonel T. R. Tempel, USA, DC, Chief of Staff, Chief of Army Dental Corps, the Pentagon, Washington, D. C.
 - Major C. Harris, MEDDAC, Fort Sheridan, Illinois.
- LT W. S. Bold, DC, USN, Naval Dental Clinic, Great Lakes, Illinois.

APRIL

- HMCM L. V. Green, Jr., USN, Force Master Chief, Naval Medical Command, Washington, D. C.
- LT R. Wisner, DC, USNR, Naval Dental Clinic, Great Lakes, Illinois.
- Dr. S. Hoff, Chief, Pharmacology Department, Chicago Medical School, North Chicago, Illinois.
- Captain D. E. Furry, MSC, USN, Executive Officer, Naval Medical Research and Development Command, Bethesda, Maryland.
- LT P. W. Tubbs, Officer in Charge, Personnel Support Activity Detachment, Naval Hospital, Great Lakes, Illinois.
- Colonel C. W. DeLannoy, USAR, VC, Department of Animal Medicine, Michael Reese Hospital, Chicago, Illinois.
- Dr. Walkmeier, Northwestern University, Dental Materials Graduate Program, Chicago, Illinois.

MAY

- Dr. J. F. Goggins, Dean, Marquette University School of Dentistry, Milwaukee, Wisconsin.
- Dr. K. C. Hoerman, Loyola University School of Dentistry, Maywood, Illinois.
- Ms. C. A. Hughes, Loyola University School of Dentistry, Maywood, Illinois.
- Mr. A. E. Helper, National Research Council, Clifton, Massachusetts.

JULY

Captain E. B. Hancock, DC, USN, Oral and Dental Health Program Manager, Naval Medical Research and Development Command, Bethesda, Maryland.

JUNE (Continued)

LCDR R. C. Rockhill, MSC, USN, Naval Medical Research and Development Command, Bethesda, Maryland.

LTjg D. M. Buck, MSC, USNR, Naval Medical Research and Development Command, Bethesda, Maryland.

AUGUST

and research, according movement

Commodore L. E. Angelo, MSC, USN, Naval Medical Command, Washington, D. C.

Captain E. B. Hancock, DC, USN, Oral and Dental Health Program Manager, Naval Medical Research and Development Command, Bethesda, Maryland.

Lieutenant Colonel B. S. Goodwin, Naval Medical Research and Development Command, Bethesda, Maryland.

Dr. F. J. Sena, Director, New Product Development, Block Drug Company, Jersey City, New Jersey.



Captain R. G. Esquire congratulating Dr. I. L. Shklair for completion of 35 years Federal Service. Dr. Shklair was awarded a certificate and pin.

OCTOBER

- Dr. M. E. Cohen received a Quality Step Increase.
- DT2 R. W. Gamble received a Letter of Appreciation from the Chairman, 1984 Navy Relief Society Fund Drive.
- DT2 R. M. Gibbs received a Letter of Appreciation from the Commanding Officer upon his departure from NDRI for duty as a student Service School Command, Great Lakes, Illinois.
 - Dr. B. L. Lamberts received a Merit Pay Performance Award.
- Captain G. M. McWalter received a Letter of Commendation from the Commanding Officer upon his retirement from active duty.
 - Mr. E. D. Pederson received a Quality Step Increase.
 - Ms. M. J. Portis received a Quality Step Increase.
 - Ms. R. C. Quiring received a Quality Step Increase.
- Commander S. A. Ralls received a Letter of Commendation from the Commanding Officer, Naval Hospital, San Diego, California.
- Lieutenant W. V. Reese reported to NDRI for duty from the Naval Dental Clinic, Norfolk, Virginia.
 - Dr. I. L. Shklair received a Merit Pay Performance Award.
 - Dr. L. G. Simonson received a Quality Step Increase.

NOVEMBER

- LCDR J. A. Benny received a Letter of Commendation from the Commanding Officer upon her retirement from active duty.
- Lieutenant M. T. Hetzer reported for duty from the Naval Dental Clinic, Pearl Harbor, Hawaii.
- Lieutenant T. R. Jones reported for duty on the staff of the Scientific Investigations Department.
- Mr. W. O. Schnurrpusch resigned from the Fiscal/Supply Division of the Administrative Department.

DECEMBER

- DT1 S. M. Benshoof received a Letter of Commendation from the Commanding Officer upon his departure from NDRI for duty at the Branch Dental Clinic, Okinawa, Japan.
 - DT1 W. F. Bruton was selected NDRI's Sailor of the Quarter.

JANUARY

- DT1 W. F. Bruton received the Navy League Award.
- DTl W. F. Bruton received a Letter of Commendation from the Commanding Officer for his selection as Sailor of the Year for the Naval Dental Research Institute.
 - DT3 M. J. Johnson was frocked to E-5.

MARCH

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- PVT D. O. Brown was promoted to E-4.
- Dr. L. G. Simonson was reelected as Program Chairman, IADR/AADR Microbiology/Immunology Group.
- Ms. S. P. Stewart joined the staff of the Fiscal/Supply Division.
 - DT3 E. L. Tuten was selected NDRI's Sailor of the Quarter.
- Ms. S. Y. Winn resigned from the Administrative Services Division.

APRIL

- DT1 W. F. Bruton received a Letter of Commendation for his nomination for the FY 1985 Naval Medical Command Shore Sailor of the Year.
 - DN L. A. Rouse received a Navy Achievement Medal.
 - Commander R. G. Esquire was frocked to the rank of Captain.
- DN M. A. Lawhorn reported for duty from the Branch Dental Clinic, Guantanamo Bay, Cuba.
- Lieutenant W. V. Reese was awarded the Navy Commendation Medal.

HONORS, AWARDS, POSITIONS HELD, CEREMONIES, STAFF ARRIVALS, DEPARTURES, AND REENLISTMENTS (Continued)

MAY

Lieutenant J. R. Kelly received a Letter of Commendation from the Commanding Officer upon his departure from NDRI for duty under instruction at Harvard School of Dental Medicine, Boston, Massachusetts.

Ms. D. A. Tucker joined the staff of the Administrative Services Division.

JUNE

DT3 G. W. Dalm was transferred to Detachment B FSSG FMFPAC, El Toro-Santa Ana, California.

DT2 M. J. Johnson was selected as NDRI's Sailor of the Quarter.

JULY

DT2 D. B. Bartelme was frocked to E-6.

DT1 W. F. Bruton received a Navy Unit Commendation Award from the 3D Force Service Support Group, Fleet Marine Force, Pacific.

LCDR M. C. Diehl reported for duty from the 3D FSSG, FMFPAC, Okinawa, Japan.

Captain R. G. Esquire received a Letter of Appreciation from the Commanding Officer, Naval Hospital, Great Lakes, Illinois for participation in the Fitness for Life Health Fair.

Lieutenant P. M. Hamilton reported for duty from the USS Enterprise.

DTCS M. G. Hastings received a Navy Unit Commendation Award from the 3D Force Service Support Group, Fleet Marine Force, Pacific.

Lieutenant T. R. Jones received an Expert Pistol Medal.

 ${\tt DT3}$ J. A. Jones received a Meritorious Unit Commendation Award for duty on the USS Orion.

DT3 J. A. Jones was frocked to E-5.

DT1 G. M. McKendall received a Good Conduct Award.

DN L. A. Rouse was frocked to E-4.

DT3 E. L. Tuten was frocked to E-5.

HONORS, AWARDS, POSITIONS HELD, CEREMONIES, STAFF ARRIVALS, DEPARTURES, AND REENLISTMENTS (Continued)

JULY (Continued)

The following personnel received Letters of Appreciation from the Andrew Cooke Magnet School for participation in the Adopt-A-School Program:

- S. R. HARMON
- M. G. HASTINGS
- B. E. JOHNSON
- R. R. ROBERSON

AUGUST

Commander R. S. Baycar received a Letter of Commendation from the Commanding Officer upon his departure for duty on the USS Shenandoah, Norfolk, Virgina.

LCDR B. A. Halverson reported for duty from the Naval Dental Clinic, Rota, Spain.

HM1 E. B. Johnson received an Expert Pistol Medal.

DT3 L. A. Rouse was advanced to E-4.

Captain R. G. Walter reported for duty from the Branch Dental Clinic, Guantanomo Bay, Cuba.

SEPTEMBER

- DT1 W. F. Bruton was advanced to E-7.
- DT1 S. R. Harmon received a Good Conduct Award.
- DT1 S. R. Harmon was selected as NDRI's Sailor of the Quarter.

Lieutenant W. V. Reese received a Navy Achievement Medal.